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In vitro kinase assays for STPKs.

Ramandeep Singh

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An abbreviated version of this protocol was published in Antimicrobial Agents and Chemotherapy in Aug 2019

NU-6027 Inhibits Growth of Mycobacterium tuberculosis by Targeting Protein Kinase D and Protein Kinase G

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Detailed protocol

The purified STPKs (Pkn A, B, D, J, L, G and K) were incubated in PIPES buffer (100 mM PIPES, pH 7.0, 80 mM NaCl and 20 mM $MgCl_2$) containing 1-2µCi of [γ-^{32}P] ATP for 10-45 minutes at 25°C. Autophosphorylation assays of PknE, PknF, and PknH were performed in buffer containing 25 mM Tris-Cl, pH 7.4, 5 mM MgCl2, 2mM MnCl2, and 1 mM DTT for 30 min at 37°C. The reaction was stopped by adding of 5X SDS sample loading buffer. To check the effect of NU 6027 (dissolved in DMSO), variable concentration (0.01-100µM) of NU-6027 was added in the reaction mixture and incubated for the same duration as control for a particular STPK. In control set DMSO was used instead of NU-6027. The effect of NU-6027 (50 & 100 µM) was checked against PknA, B, D, G and K However, the autophosphorylation activity inhibition of PknE, F, H, J and L was performed against 50mM concentration of NU-6027. Proteins were resolved on 12%SDS-PAGE. After electrophoresis, the gel was wrapped in cellophane sheet and exposed to a phosphor imaging plate for 12 hours followed by scanning with Typhoon 9210 imager. The gel was then stained with Commassie R_{250} to visualize the protein bands. Image J was used to calculate the intensity of autophosphorylation of Pkns (with and without inhibitor) and Ic50 was calculated using GraphPad Prism7.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Singh, R. (2021). In vitro kinase assays for STPKs.. Bio-protocol Preprint. bio-protocol.org/prep1231.
2. Kidwai, S., Bouzeyen, R., Chakraborti, S., Khare, N., Das, S., Gosain, T. P., Behura, A., Meena, C. L., Dhiman, R., Essafi, M., Bajaj, A., Saini, D. K., Srinivasan, N., Mahajan, D. and Singh, R.(2019). NU-6027 Inhibits Growth of Mycobacterium tuberculosis by Targeting Protein Kinase D and Protein Kinase G. Antimicrobial Agents and Chemotherapy 63(9). DOI: 10.1128/AAC.00996-19